

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

In the claims

Claim 1 (withdrawn): A method of enhancing cardiac function in a mammal, comprising delivering a vector to the heart of said mammal, the vector comprising a gene encoding a beta-adrenergic signaling protein (beta-ASP) operably linked to a promoter.

Claim 2 (withdrawn): A method of enhancing cardiac function according to claim 1, wherein the vector is introduced into a blood vessel supplying blood to the myocardium of the heart.

Claim 3 (withdrawn): A method of enhancing cardiac function according to claim 1, wherein the vector is delivered to cardiac myocytes.

Claim 4 (withdrawn): A method of enhancing cardiac function according to claim 2, wherein said blood vessel supplying blood to the myocardium of the heart is a coronary artery, a saphenous vein graft or an internal mammary artery graft.

Claim 5 (withdrawn): A method of enhancing cardiac function according to claim 4, wherein the vector is introduced into both left and right coronary arteries.

Claim 6 (withdrawn): A method of enhancing cardiac function according to claim 1, wherein said mammal is a human.

Claim 7 (withdrawn): A method of enhancing cardiac function according to claim 1, wherein the vector comprises a gene encoding a beta-ASP selected from the group consisting of a beta-adrenergic receptor (beta-AR), a G-protein receptor kinase inhibitor (GRK inhibitor) and an adenylylcyclase (AC).

Claim 8 (withdrawn): A method of enhancing cardiac function according to claim 1, wherein the vector comprises genes encoding two different beta-adrenergic signaling proteins operably linked to a promoter.

Claim 9 (withdrawn): A method of enhancing cardiac function according to claim 1, further comprising introducing a second vector comprising a gene encoding a second beta-ASP operably linked to a promoter, wherein said second beta-ASP is different from said first beta-ASP.

Claim 10 (withdrawn): A method of enhancing cardiac function according to claim 7, wherein the beta-ASP is selected from the group consisting of a beta₁-adrenergic receptor (beta₁-AR) and a beta₂-adrenergic receptor (beta₂-AR).

Claim 11 (withdrawn): A method of enhancing cardiac function according to claim 10, wherein the beta-ASP is a beta₁-adrenergic receptor (beta₁-AR).

Claim 12 (withdrawn): A method of enhancing cardiac function according to claim 7, wherein the gene encoding a beta-ASP is a gene encoding a GRK inhibitor.

Claim 13 (withdrawn): A method of enhancing cardiac function according to claim 7, wherein the beta-ASP is an adenylylcyclase (AC).

Claim 14 (withdrawn): A method of enhancing cardiac function according to claim 13, wherein the beta-ASP is AC isoform VI.

Claim 15 (withdrawn): A method of enhancing cardiac function according to claim 14, wherein the AC isoform VI comprises the amino acid sequence of SEQ ID NO. 13.

Claim 16 (withdrawn): A method of enhancing cardiac function according to claim 15, wherein the gene encoding the AC isoform VI comprises the nucleotide sequence of SEQ ID NO. 12.

Claim 17 (withdrawn): A method of enhancing cardiac function according to claim 13, wherein the beta-ASP is human AC isoform VI.

Claim 18 (withdrawn): A method of enhancing cardiac function according to claim 13, wherein the beta-ASP is the human AC isoform VI of SEQ ID NO. 11.

Claim 19 (withdrawn): A method of enhancing cardiac function according to claim 7, wherein the gene encoding a beta-ASP is operably linked to a heterologous promoter selected from the group consisting of a heterologous constitutive promoter and a heterologous inducible promoter.

Claim 20 (withdrawn): A method of enhancing cardiac function according to claim 19, wherein the promoter is selected from the group consisting of a ventricular myosin light chain 2 promoter and a ventricular myosin heavy chain promoter.

Claim 21 (withdrawn): A method of enhancing cardiac function according to claim 19, wherein the gene encoding a beta-ASP is a gene encoding AC isoform VI operably linked to a heterologous promoter.

Claim 22 (withdrawn): A method of enhancing cardiac function according to claim 19, wherein the gene encoding a beta-ASP is the gene of SEQ ID NO. 10 encoding human AC isoform VI operably linked to a heterologous promoter.

Claim 23 (withdrawn): A method of enhancing cardiac function according to claim 19, wherein the gene encoding a beta-ASP is a modified AC isoform VI gene operably linked to a heterologous promoter.

Claim 24 (withdrawn): A method of enhancing cardiac function according to claim 23, wherein the modified AC isoform VI gene encodes a polypeptide comprising the amino acid sequence of SEQ ID NO. 13.

Claim 25 (withdrawn): A method of enhancing cardiac function according to claim 1, wherein the gene encoding a beta-ASP is a variant of a wild-type beta-ASP gene, the variant comprising a deletion in one or more untranslated regions of said beta-ASP gene.

Claim 26 (withdrawn): A method of enhancing cardiac function according to claim 25, wherein said deletion removes at least about 100 bp of the 3'-untranslated region.

Claim 27 (withdrawn): A method of enhancing cardiac function according to claim 25, wherein the gene encoding a beta-ASP is a variant AC gene having a deletion in the 3'-untranslated region.

Claim 28 (withdrawn): A method of enhancing cardiac function according to claim 25, wherein the gene encoding a beta-ASP is a truncated AC_{VI} gene having a deletion removing the 3'-untranslated region.

Claim 29 (withdrawn): A method of enhancing cardiac function according to claim 1, wherein the vector is selected from the group consisting of a viral vector and a lipid-based vector.

Claim 30 (withdrawn): A method of enhancing cardiac function according to claim 1, wherein the vector is a viral particle.

Claim 31 (withdrawn): A method of enhancing cardiac function according to claim 30, wherein the viral particle is selected from the group consisting of an adenovirus (Ad) and an adeno-associated virus (AAV).

Claim 32 (withdrawn): A method of enhancing cardiac function according to claim 31, wherein the viral particle is an adenovirus comprising a polynucleotide having a promoter operably linked to a gene encoding a beta-ASP, and said adenovirus vector is replication-defective in humans.

Claim 33 (withdrawn): A method of enhancing cardiac function according to claim 32, wherein the beta-ASP is an adenylylcyclase (AC) isoform VI.

Claim 34 (withdrawn): A method of enhancing cardiac function according to claim 32, wherein the beta-ASP is a modified AC isoform VI of SEQ ID NO. 13.

Claim 35 (withdrawn): A method of enhancing cardiac function according to claim 32, wherein the beta-ASP is the human AC isoform VI of SEQ ID NO. 11.

Claim 36 (withdrawn): A recombinant replication-defective viral particle comprising a gene encoding a beta-ASP operably linked to a promoter.

Claim 37 (withdrawn): A recombinant replication-defective viral particle according to claim 36, wherein said promoter is a heterologous promoter.

Claim 38 (withdrawn): A recombinant replication-defective viral particle according to claim 36, wherein said beta-ASP is selected from the group consisting of a beta-AR, a GRK inhibitor and an adenylylcyclase.

Claim 39 (withdrawn): A recombinant replication-defective viral particle according to claim 36, wherein the vector comprises genes encoding two different beta-ASPs.

Claim 40 (withdrawn): A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is a beta₁-adrenergic receptor (beta₁-AR).

Claim 41 (withdrawn): A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is selected from the group consisting of AC isoform II, AC isoform V and AC isoform VI.

Claim 42 (withdrawn): A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is AC isoform VI.

Claim 43 (withdrawn): A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is a chimeric AC.

Claim 44 (withdrawn): A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is the AC isoform VI of SEQ ID NO. 13.

Claim 45 (withdrawn): A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is human AC isoform VI.

Claim 46 (withdrawn): A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is human AC isoform VI of SEQ ID NO. 11.

Claim 47 (withdrawn): A recombinant replication-defective viral particle according to claim 36, wherein the gene encoding a beta-ASP is a variant of a wild-type beta-ASP gene, the variant comprising a deletion in one or more untranslated regions of said beta-ASP gene.

Claim 48 (withdrawn): A recombinant replication-defective viral particle according to claim 47, wherein said deletion removes at least about 100 bp of the 3'-untranslated region.

Claim 49 (withdrawn): A recombinant replication-defective viral particle according to claim 47, wherein the gene encoding a beta-ASP is a variant AC gene having a deletion in the 3'-untranslated region.

Claim 50 (withdrawn): A recombinant replication-defective viral particle according to claim 47, wherein the gene encoding a beta-ASP is a truncated AC_{VI} gene having a deletion removing the 3'-untranslated region.

Claim 51 (withdrawn): A recombinant replication-defective viral particle according to claim 36, wherein said recombinant replication-defective viral particle is an adenovirus that is replication-defective in humans.

Claim 52 (withdrawn): A mammalian cell transfected with a recombinant replication-defective viral particle according to claim 36.

Claim 53 (withdrawn): A filtered adenovirus particle preparation comprising:

- (i) a recombinant replication-defective adenovirus particle according to claim 36, and
- (ii) a carrier.

Claim 54 (withdrawn): A filtered injectable adenovirus particle preparation according to claim 53, wherein said adenovirus vector has been filtered through a 0.1-0.5 micron filter.

Claim 55 (withdrawn): A method of generating a recombinant replication-defective viral particle according to claim 36, comprising the following steps in the order listed:

- (i) introducing first and second plasmids into a replication-permissive mammalian cell expressing one or more adenovirus genes conferring replication competence, wherein said first plasmid comprises a gene encoding a beta-ASP operably linked to a promoter and further comprises a replication-defective human adenovirus genome, and wherein said second plasmid comprises a replication-proficient human adenovirus genome and further comprises an additional polynucleotide sequence making the second plasmid too large to be encapsidated in an adenovirus particle, whereby rescue recombination takes place between the first plasmid and the second plasmid to generate a recombinant adenoviral genome comprising the gene encoding a beta-ASP but lacking one or more adenoviral replication genes, said recombinant genome being sufficiently small to be encapsidated in an adenovirus particle;
- (ii) identifying successful recombinant viral vectors in cell culture; and
- (iii) propagating a resulting recombinant viral particle in replication-permissive mammalian cells expressing the missing adenoviral replication genes to generate a recombinant replication-defective viral particle.

Claim 56 (withdrawn): A method of generating a viral particle according to claim 55, wherein said identification step comprises the steps of:

- (i) monitoring transfected cells for evidence of cytopathic effect;
- (ii) isolating viral nucleic acid from the cell supernatant of cultures of the transfected cells showing a cytopathic effect;
- (iii) identifying successful recombinant viral vectors by PCR using primers complementary

to the promoter operably linked to the beta-ASP gene and primers complementary to adenovirus sequences; and

(iv) purifying the recombinant viral particles by plaque purification.

Claim 57 (withdrawn): A recombinant pro-viral plasmid comprising a gene encoding a beta-ASP operably linked to a promoter and further comprising a replication-defective viral genome.

Claim 58 (withdrawn): A recombinant pro-viral plasmid according to claim 57, wherein said beta-ASP is selected from the group consisting of a beta-AR, a GRK inhibitor and an adenylylcyclase.

Claim 59 (withdrawn): A recombinant pro-viral plasmid according to claim 57, wherein said beta-ASP is adenylylcyclase isoform VI.

Claim 60 (withdrawn): A recombinant pro-viral plasmid according to claim 57, wherein said beta-ASP is a chimeric adenylylcyclase.

Claim 61 (withdrawn): A recombinant pro-viral plasmid according to claim 57, wherein said beta-ASP is the adenylylcyclase isoform VI of SEQ ID NO. 13.

Claim 62 (withdrawn): A recombinant pro-viral plasmid according to claim 57, wherein said beta-ASP is adenylylcyclase human isoform VI.

Claim 63 (withdrawn): A recombinant pro-viral plasmid according to claim 57, wherein said beta-ASP is the adenylylcyclase human isoform VI of SEQ ID 11.

Claim 64 (withdrawn): A recombinant pro-viral plasmid according to claim 57, wherein the gene encoding a beta-ASP is a variant of a wild-type beta-ASP gene, the variant comprising a deletion in one or more untranslated regions of said beta-ASP gene.

Claim 65 (withdrawn): A recombinant pro-viral plasmid according to claim 64, wherein said deletion removes at least about 100 bp of the 3'-untranslated region.

Claim 66 (withdrawn): A recombinant pro-viral plasmid according to claim 64, wherein the gene encoding a beta-ASP is a variant AC gene having a deletion in the 3'-untranslated region.

Claim 67 (withdrawn): A recombinant pro-viral plasmid according to claim 64, wherein the gene encoding a beta-ASP is a truncated AC_{VI} gene having a deletion removing the 3'-untranslated region.

Claim 68 (withdrawn): A recombinant pro-viral plasmid according to claim 57, wherein said replication-defective viral genome is a replication-defective adenoviral genome.

Claim 69 (withdrawn): A cell comprising a recombinant pro-viral plasmid according to claim 57.

Claim 70 (withdrawn): A polynucleotide comprising a sequence encoding a chimeric adenylylcyclase polypeptide.

Claim 71 (withdrawn): A polynucleotide of claim 70 encoding the AC_{VI} of SEQ ID NO. 13.

Claim 72 (withdrawn): A polynucleotide of claim 70 comprising the nucleotide sequence of SEQ ID NO. 12.

Claim 73 (cancelled)

Claim 74 (currently amended): An isolated polynucleotide comprising a nucleic acid sequence encoding [[a]] the human adenylylcyclase VI (AC_{VI}) polypeptide of SEQ ID NO. 11.

Claims 75-78 (cancelled)

Claim 79 (withdrawn): An isolated polypeptide encoded by the polynucleotide of claim 70.

Claim 80 (withdrawn): An isolated polypeptide encoded by the polynucleotide of claim 71.

Claim 81 (withdrawn): An isolated polypeptide encoded by the polynucleotide of claim 72.

Claim 82 (withdrawn): An isolated polypeptide encoded by the polynucleotide of claim 73.

Claim 83 (withdrawn): An isolated polypeptide encoded by the polynucleotide of claim 74.

Claim 84 (withdrawn): An isolated polypeptide of claim 82, wherein said polypeptide comprises a sequence of at least 300 amino acid residues that has at least 95% overall amino acid sequence identity with a sequence of comparable length within the sequence shown in SEQ ID NO. 2 or 4 or 6.

Claim 85 (withdrawn): An isolated polypeptide of claim 84, wherein said overall amino acid sequence identity is at least 99%.

Claim 86 (withdrawn): A vector comprising a polynucleotide of claim 70.

Claim 87 (withdrawn): A vector comprising a polynucleotide of claim 71.

Claim 88 (withdrawn): A vector comprising a polynucleotide of claim 72.

Claim 89 (cancelled)

Claim 90 (currently amended): A vector comprising ~~[[a]]~~ the polynucleotide of claim 74.

Claim 91 (withdrawn): A vector of claim 86, wherein said vector is selected from the group consisting of a viral vector and a lipid-based vector.

Claim 92 (withdrawn): A vector of claim 86, wherein said vector is a replication-defective viral vector selected from the group consisting of an adenoviral vector and an adeno-associated viral vector.

Claim 93 (withdrawn): A vector of claim 87, wherein said vector is selected from the group consisting of a viral vector and a lipid-based vector.

Claim 94 (withdrawn): A vector of claim 87, wherein said vector is a replication-defective viral vector selected from the group consisting of an adenoviral vector and an adeno-associated viral vector.

Claim 95 (withdrawn): A vector of claim 88, wherein said vector is selected from the group consisting of a viral vector and a lipid-based vector.

Claim 96 (withdrawn): A vector of claim 88, wherein said vector is a replication-defective viral vector selected from the group consisting of an adenoviral vector and an adeno-associated viral vector.

Claim 97 (cancelled)

Claim 98 (cancelled)

Claim 99 (currently amended): [[A]] The vector of claim 90, wherein said vector is selected from the group consisting of a viral vector, and a ~~lipid-based~~ liposome vector.

Claim 100 (currently amended): [[A]] The vector of claim 90, wherein said vector is a replication-defective viral vector selected from the group consisting of an adenoviral vector, and an adeno-associated viral vector.

Claim 101 (withdrawn, currently amended): [[An]] The isolated polynucleotide of claim 74, comprising the nucleotide sequence of SEQ ID NO: 10.